# Tat and Trans-activation-responsive (TAR) RNA-independent Induction of HIV-1 Long Terminal Repeat by Human and Murine Cyclin T1 Requires Sp1*

### Venkat S. R. K. Yedavalli‡, Monsef Benkirane§, and Kuan-Teh Jeang‡¶

*From the ‡Molecular Virology Section, Laboratory of Molecular Microbiology, National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, Maryland 20892-0460 and §Laboratoire de Virologie Moléculaire et Transfert de Gène, Institut de Génétique Humaine, CNRS Unité Propre de Recherche 1142, 34296 Montpellier, France*

P-TEFb, cyclin T1 + CDK9, is needed for the expression of cellular promoters and primate lentiviral long terminal repeats (LTRs). Curiously, cellular and lentiviral promoters differ dramatically in the requirements for positive transcriptional elongation factor (P-TEF) b activity. Lentiviral LTRs, but not cellular promoters, need an RNA-associated P-TEFb/Tat/TAR (trans-activation-responsive) RNA ternary complex. Ternary complex defective murine cycT1 is apparently inactive for lentiviral transcription. Why P-TEFb requires Tat/TAR for LTRs but not for cellular promoters remains unknown. To explore this question, we sought to determine whether DNA targeting of murine and human cyclin T1 can reconstitute a Tat/TAR-independent activity to the HIV-1 LTR. In the absence of Tat and TAR, we found that both HuCycT1 and MuCycT1 can robustly activate the HIV-1 LTR. We further showed that Sp1 is necessary and sufficient for this DNA-targeted activity. Thus, like cellular promoters, HIV-1 LTR can use P-TEFb function without a Tat/TAR RNA complex. This activity could explain recent findings of robust HIV-1 replication in rat cells that cannot form a P-TEFb/Tat/TAR moiety.

Human immunodeficiency virus 1 (HIV-1)[1] transcription is an excellent paradigm for studying RNA polymerase II (RNAP II) elongation (1). HIV-1 encodes a transactivator protein, Tat, that stimulates transcription elongation through interaction with a trans-activation-responsive element (TAR) located at the 5′ end of the nascent transcripts (2). Tat has been suggested to recruit an RNAP II C-terminal domain (CTD) kinase to the HIV-1 promoter (3–6). Hyperphosphorylation of RNAP II CTD by this kinase correlates closely with productive transcriptional elongation (5, 7, 8). The Tat-associated CTD-kinase has been shown to comprise cdc2-related kinase (PITALRE/CDK9) (9, 10) and cyclin (cyclin T1) (11, 12). CDK9 and cyclin T1 (cycT1) are components of previously identified positive

transcriptional elongation factor (P-TEFb) in *Drosophila melanogaster* and mammals (10, 13, 14). Our current understanding is that P-TEFb forms a ternary complex with Tat and TAR to mediate transcriptional elongation from the HIV-1 LTR. Hence, mouse cycT1, which differs from human cycT1 by a cysteine at position 261, cannot form a P-TEFb/Tat/TAR complex; this defect is presumed to explain the long-observed defect in HIV-1 transcription in rodent cells (12, 15–17).

Recent findings suggest that P-TEFb also plays an essential role in the expression of mammalian and *D. melanogaster* genes (10, 13, 14, 18, 19). In these settings, P-TEFb must engage promoter-DNA independently of TAR RNA and must promote processive transcription without Tat. Indeed, it is a mystery why the cellular-promoter operative activity of P-TEFb does not apparently apply to the HIV-1 LTR. Here, we sought to determine whether DNA targeting of murine or human cycT1 to the HIV-1 LTR can bypass a requirement for Tat/TAR. We show that both murine and human cycT1 can interact with the Sp1 "A" domain to reconstitute Tat/TAR independent transcriptional activity and that Sp1 is necessary and sufficient to recruit cycT1 to the HIV-1 LTR. These results support the finding of P-TEFb in HIV-1 preinitiation complexes before the synthesis of TAR RNA (20) and help to explain the normal expression of HIV-1 LTR in rat cells that cannot form an RNA-bound P-TEFb (21, 22). Thus, we propose that lentiviral LTRs, like cellular promoters, can use P-TEFb despite absence of Tat/TAR. This proposition concurs with recent suggestions that successful promoter recruitment of cycT1 is fully sufficient for HIV-1 LTR transcription (23).

## EXPERIMENTAL PROCEDURES

*Plasmids*—pCMV-HuCycT1 and pCMV-MuCycT1 (1–708) were kindly provided by Drs. David Price and Kathy Jones, respectively (11, 24). Full-length MuCycT1 and HuCycT1 were reconstructed by PCR. pCMV-HA (Clontech) and pSG424 (Gal4 binding domain) vectors were used for cyclin T1 fusion vectors. p(−)43 chloramphenicol acetyltransferase (25), HIV-1 LTR luciferase plasmids (26), Gal4Sp1A (27), and G5-83HIV-Luc (21) have been described previously. SIVsykes and SIVmac LTR-luciferase plasmids were cloned into pGL3-basic (Promega, Madison, WI). HIV-1 linker scanning mutants were from AIDS reference reagent program donated by Dr. Steven Zeichner (26).

*Cell Culture, Transfection, and Reporter Assays*—Cell propagation, transfection, and reporter assays were as described previously (28). All transfections were repeated three or more times and were normalized to β-galactosidase activity expressed from a cotransfected pCMV-β (Clontech). Average values ± S.D. are shown. In all experiments, we have assayed both the HuCycT1 and the equivalent MuCycT1 plasmids. Because of space limitations, only one or the other set of results is shown.

*Antibodies*—Mouse monoclonal anti-HA (Sigma Chemical); mouse monoclonal anti-Gal4BD (Santa Cruz Biotechnology, Santa Cruz, CA) rabbit polyclonal anti-Sp1 and anti-HA antibody (Upstate Biotechnology, Lake Placid, NY) were used as described.

[1] The abbreviations used are: HIV-1, human immunodeficiency virus type 1; RNAP II, RNA polymerase II; TAR, trans-activation-responsive element; CTD, C-terminal domain; P-TEFb, positive transcriptional elongation factor; cycT1, cyclin T1; CMV, cytomegalovirus; HA, hemagglutinin; NF-κB, nuclear factor-κB; SIV, simian immunodeficiency virus; MuCycT1, murine cyclin T1; HuCycT1, human cyclin T1; AP-1, activator protein 1; NFAT, nuclear factor of activated T cells; Gal4BD, Gal4 DNA binding domain.

**This paper is available on line at http://www.jbc.org**

FIG. 1. **HuCycT1 and MuCycT1 activate lentiviral LTRs in the absence of Tat.** *A*, schematic representations of human (HIV-1 HXB2) and simian (SIVmac and SIVsykes) LTR-luciferase reporters. HIV-1, SIVmac, and SIVsykes LTRs have two, one, and zero NF-$\kappa$B binding sites, respectively. *B*, HeLa cells were transfected with 200 ng of HIV-1 HXB2 LTR (*lanes 1–3*), SIVmac LTR (*lanes 4–6*) or SIVsykes (*lanes 7–9*) LTR-luciferase plasmid with 2.0 $\mu$g of either HuCycT1 or MuCycT1. Transfections were normalized to cotransfected pCMV-$\beta$-galactosidase plasmid. *C*, dose-titrated activity of MuCycT1. HeLa cells were cotransfected with 200 ng of either HIV-1 HXB2 or SIVsykes LTR-luciferase plasmid with increasing amounts of MuCycT1. All transfections were normalized to cotransfected pCMV-$\beta$. All values are averages ± S.D. from three or more independent experiments.
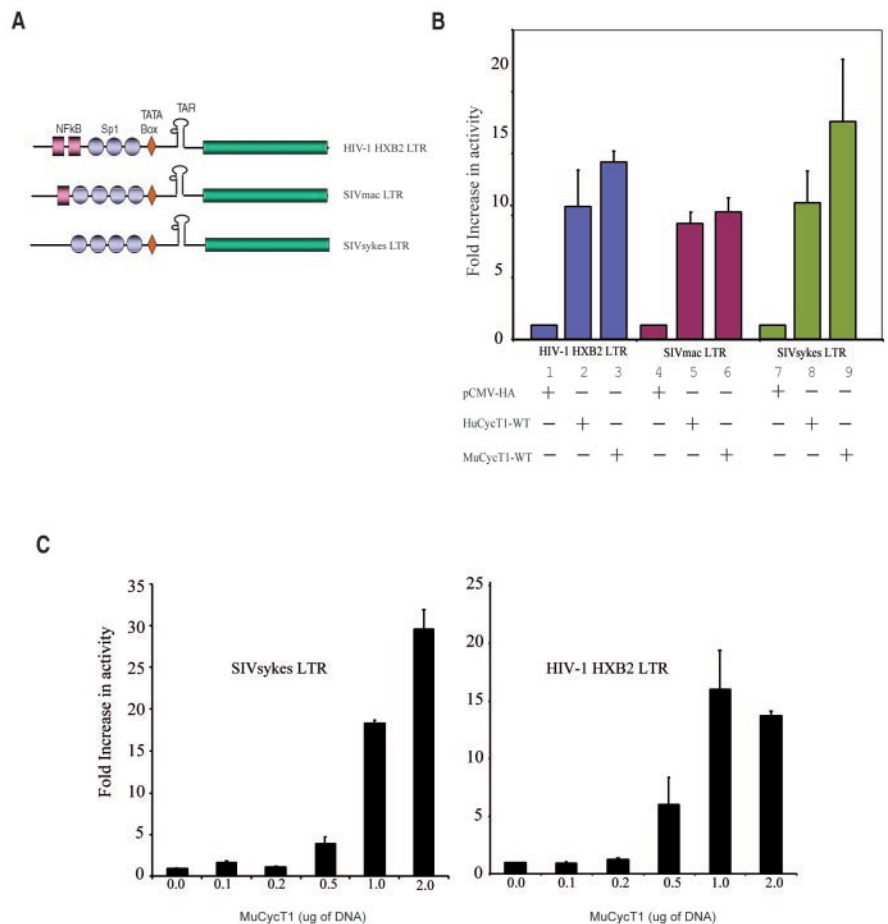
*Western Blotting, Immunoprecipitation, and Confocal Imaging—*Western blotting and immunoprecipitation were performed as described previously (28). For confocal microscopy, HeLa cells were cultured on 25-mm coverslips (Thomas Scientific) and transfected with plasmid DNA. One day later, cells were fixed with 3.7% formaldehyde, permeabilized with PBS containing 0.1% Triton X-100, and incubated with monoclonal anti-HA antibody followed with anti-mouse conjugated to Alexa Fluor 488 (Molecular Probes, Eugene, OR). Nuclei were stained with 4,6-diamidino-2-phenylindole (Molecular Probes). Coverslips were mounted onto glass slides with ProLong antifade kit (Molecular Probes) and examined with a Leica laser-scanning microscope.

## RESULTS

*Robust Activation of Primate Lentiviral LTRs by cycT1 in the Absence of Tat—*The great preponderance of HIV-related P-TEFb studies have focused on transcriptional elongation in the context of a ternary P-TEFb/Tat/TAR RNA complex (20, 24, 29). Recent findings, however, demonstrate that P-TEFb activates numerous cellular promoters in Tat/TAR independent settings (18, 30). Moreover, P-TEFb is in HIV-1 preinitiation complexes before the synthesis of TAR RNA (20, 31), suggesting a role distinct from its Tat/TAR RNA-bound form. Prompted by these observations, we sought to investigate the impact of P-TEFb on lentiviral LTR-expression in the absence of Tat/TAR.

Human and subhuman primate lentiviral LTRs conserve biological function (32) without preserving sequence identity. For example, although three or more copies of Sp1 binding motifs are found in all primate lentiviral LTRs, NF-$\kappa$B-binding sites are sporadically reduced in number or absent altogether in simian immunodeficiency (SIV) viral LTRs (Fig. 1A). Using three different primate LTRs, HIV-1 LTR (HXB2), and SIV LTRs (SIVmac and SIVsykes), we asked how each responds to trans-expression of either human cyclinT1 (HuCycT1) or mu-

rine cyclinT1 (MuCycT1). When the appropriate LTR-luciferase reporters were cotransfected into HeLa cells with either HuCycT1-alone (Fig. 1*B*, *lanes 2*, *5*, *8*) or MuCycT1-alone (Fig. 1*B*, *lanes 3*, *6*, and *9*), we observed strong activation of all LTRs. Previously, the salient difference between HuCycT1 and MuCycT1 was that the latter cannot form a functional P-TEFb/Tat/TAR ternary complex (20, 24, 29). Interestingly, the current Tat-independent assays revealed equivalent activities for both cyclin T1s. Dose-dependent titrations further confirmed that MuCycT1 activates expression effectively from NF-$\kappa$B-containing and NF-$\kappa$B-absent LTRs (Fig. 1*C*).

*Requirement for Sp1 in cycT1-mediated LTR Activation—*The HIV-1 LTR contains several transcription factor binding sites, including AP-1, NFAT, NF-$\kappa$B, Sp1, and TAR. Results in Fig. 1*A* suggest that NF-$\kappa$B is not needed for cycT1-activation. To address the other motifs in the HIV-1 LTR, we employed several, otherwise isogenic, linker-scanning LTR-mutants (Fig. 2*A*). In parallel assays, we found that the Sp1 motifs were strictly required for cycT1 activity, whereas AP-1, NFAT, NF-$\kappa$B, or TAR sequences were dispensable (Fig. 2*A*). Hence, although Tat-dependent activity of cycT1 requires TAR, the Tat-independent activity does not.

To better understand the Sp1 requirement, we explored the possibility that Sp1 and cycT1 directly interact. We transfected HA-tagged HuCycT1 or MuCycT1 into HeLa cells and immunoprecipitated mock- (*lanes 1* and *5*), HuCycT1- (*lanes 1*, *2*, *6*, *7*), or MuCycT1- (*lanes 4* and *8*) transfected cells with either anti-HA (*lanes 1–4*) or an irrelevant isotype-matched control serum (*lanes 5–8*). Immunoprecipitates were then analyzed by Western blotting for corecovery of HeLa cell-endogenous Sp1. Consistent with their similar functional activities (Fig. 2*A*), both human and murine CycT1 bound Sp1.

A



FIG. 2. **Requirement for Sp1 binding sites in cycT1 mediated LTR-activation.** *A*, HIV-1 LTR linker scanning mutants with selective mutation in AP1 (*lane 1*-3), NF-AT (*lanes 4–6*), TAR (*lanes 7–9*), NF-κB (*lanes 10–12*), or Sp1 (*lanes 13–15*), were transfected with either HuCycT1 or MuCycT1. *B*, HeLa cells transfected with HA-tagged HuCycT1 and MuCycT1 were lysed and immunoprecipitated with mouse monoclonal anti-HA antibody (*lanes 1–4*) or an irrelevant isotype-matched control serum (*lanes 5–9*). Immunoprecipitates (*IP*) were solubilized and probed in Western blotting (*WB*) with rabbit anti-Sp1 antibody. Control represents transfection with pCMV-HA. *Lane 9* shows HeLa cell extract.

B
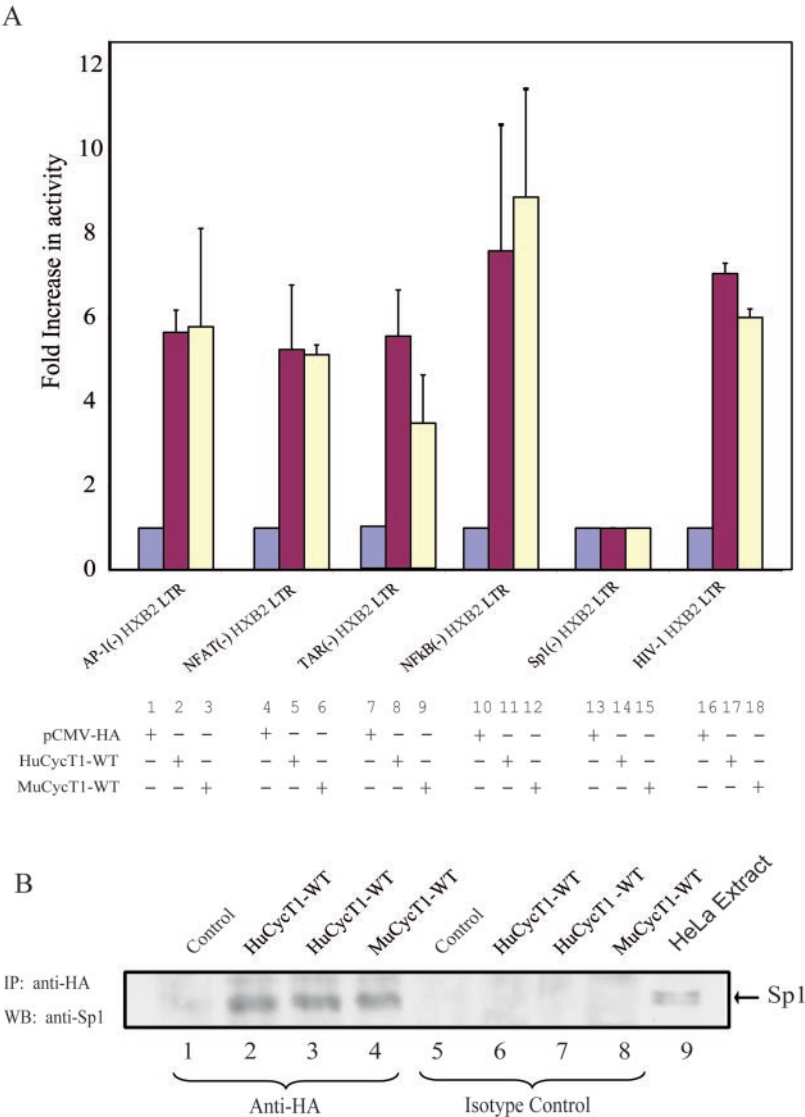


*The Sp1 A Domain Suffices for Functional and Physical Interaction with cycT1*—Sp1 is a 95- to 105-kDa transcription factor that binds DNA through its C-terminal zinc fingers (33, 34). Previously, it was shown that Sp1 contributes importantly to the expression of the HIV-1 LTR (35) and that its serine/threonine- and glutamine-rich "A" activation domain suffices for functional interaction with Tat (27, 36). These observations together with above results (Fig. 2) lead us to ask whether the functional interaction between cycT1 and Sp1 is entirely circumscribed in the A domain. To address this, we transfected HeLa cells with a chloramphenicol acetyltransferase reporter (G3(−)43CAT) composed of three Gal4 binding sites positioned upstream of a TATA box with a downstream chloramphenicol acetyltransferase cDNA (Fig. 3A). To this reporter, we separately added Gal4BD-, Gal4Sp1A-, or Gal4E1a-plasmid expressing respectively the Gal4-DNA-binding-domain, a binding-domain-Sp1A-fusion, or a binding-domain adenovirus E1a fusion. These DNA mixes were cotransfected with or without a MuCycT1 expression vector (Fig. 3B). We found that neither Gal4BD (Fig. 2B, *lanes 1* and *2*) nor Gal4E1a (Fig. 2B, *lanes 5* and *6*) supported MuCycT1-activated expression over basal. By contrast Gal4Sp1A plus MuCycT1 mediated a >70-fold increase in expression (Fig. 2B, *lanes 3* and *4*), consistent with the Sp1A segment being wholly sufficient for function.

To confirm whether Sp1A-alone was sufficient to bind CycT1

(Fig. 2B), we checked coimmunoprecipitations of Gal4Sp1A and CycT1, cyclin H, or cyclin K (Fig. 3C). HeLa cells were transfected with Gal4Sp1A and HA-tagged MuCyCT1 (*lane 1*), MuCycT1 (1–631; *lane 2*), Hu cyclin H (*lane 3*), or Hu cyclin K (*lane 4*). Gal4Sp1A was captured with anti-Gal4BD serum, and coassociated proteins were probed by Western blotting with anti-HA. We found that wild-type MuCycT1 (closely migrating as a doublet with a background band; *lane 1*), MuCycT1 (1–631; *lane 2*), and cyclin K (*lane 4*) bound Sp1A. Cyclin H (*lane 3*) did not. These results agree with cyclin K providing transcriptional roles partially redundant with cyclin T1 (30, 37) and cyclin H serving functions distinct from cyclin T1 (38).

*The C Terminus of Cyclin T1 Interacts with Sp1A to Functionally Activate Expression*—Human cyclin T1 is a 726-amino acid protein. Its N-terminal 1–290 amino acids contain a "cyclin homology box." This "box" binds cdk9 (39–41) and mediates physical interaction with HIV-1 Tat. Indeed, a single cysteine at position 261 of HuCycT1 is critical for forming a human P-TEFb/Tat/TAR complex (12, 17). Above, our results indicate a cycT1-Sp1 activity distinct from that of P-TEFb/Tat/TAR. We next examined whether the former uses a region of cycT1 different from that of the latter.

We generated several HA-tagged HuCycT1 and MuCycT1 deletion mutants (Fig. 4). Each was challenged in expression assays with cotransfected Gal4BD or Gal4Sp1A (Fig. 4). All
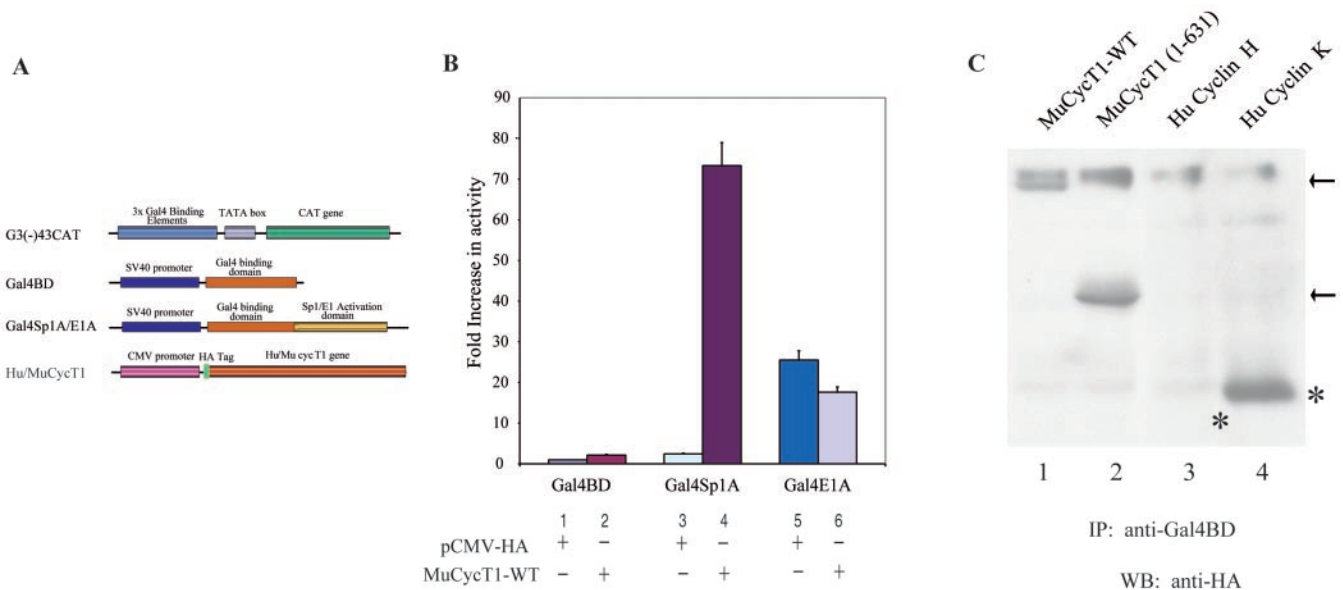
FIG. 3. **Sp1A domain sufficiently mediates cycT1 activation.** *A*, schematic representations of G3(−)43CAT, Gal4BD, Gal4Sp1A, Gal4E1A, and Hu/MuCycT1 vectors. *B*, HeLa cells were transfected with G3(−)43CAT and Gal4BD Gal4Sp1A, or Gal4E1A with or without MuCycT1. *C*, Gal4Sp1A was transfected into HeLa cells separately with HA-tagged MuCycT1 (wild-type or 1–631 mutant), HA-tagged human cyclin H, or HA-tagged human cyclin K expression vectors. Cells were immunoprecipitated with monoclonal anti-Gal4BD antibody followed by Western blotting (*WB*) with rabbit anti-HA. *Arrows*, expected positions for MuCycT1s; *asterisks*, expected positions for cycH and cycK. *IP*, immunoprecipitates.
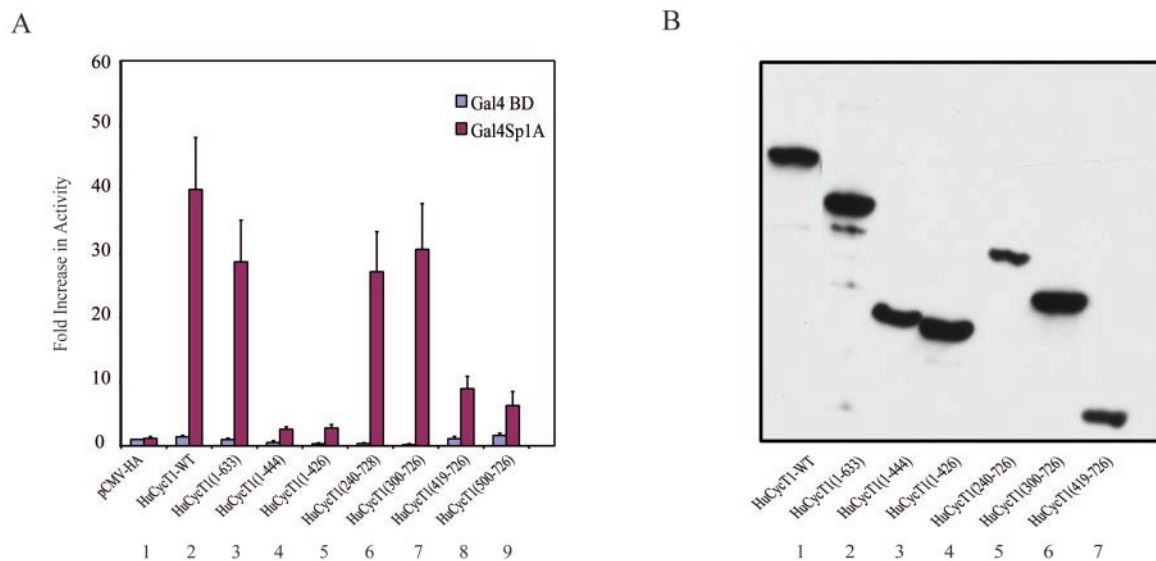


FIG. 4. **The C terminus of cycT1 cooperates with Sp1A.** *A*, HeLa cells were cotransfected with G3(−)43CAT plus either Gal4Sp1A or Gal4BD with the indicated full-length or deletion mutant of HuCycT1. *B*, Western blot verification of the expression of transfected cyclin T1s.

deletion mutants expressed comparably in HeLa cells (Fig. 4*B*), but not all supported activated expression. Thus, HuCycT1-wild type, HuCycT1 (1–633), HuCycT1 (240–726), and HuCycT1 (300–726) produced high (≥30-fold increase) activity; HuCycT1 (419–726) and HuCycT1 (500–726) provided moderate (7–10-fold increase) activity; and HuCycT1 (1–444) and HuCycT1 (1–426) were inactive (Fig. 4*A*). From the results, we mapped a Sp1A cooperative domain to the C-terminal 300 amino acids of cycT1. This C-terminal region contrasts with its N-terminal cyclin homology box, which serves cdk9-binding and P-TEFb/Tat/TAR function (24, 39).

To verify that the C-terminal region of cycT1 is discretely sufficient for transcription, we fused a sub-portion, amino acids 419 to 726, to Gal4BD. Gal4HuCycT1 (419–726) when targeted to promoter upstream Gal4-binding sites was sufficient for activating promoter expression (Fig. 5). Surprisingly, in paral-

lel assays, Gal4HuCycT1 (1–444) and Gal4HuCycT1 (1–426), previously shown by others to be competent for cdk9-binding and transcriptional elongation when bound to Tat/TAR RNA, were inactive when targeted to DNA via Gal4-binding sites (Fig. 5, *left*). Because all Gal4HuCycT1 chimeras were expressed comparably (Fig. 5, *right*), one interpretation of these results is that simple recruitment of cdk9 insufficiently supports transcription from the HIV-1 promoter.

*Sp1A-active HuCycT1s Localize to Nuclear Speckles*—Fig. 4 defined forms of HuCycT1 that are highly active, moderately active, and inactive for cooperation with Sp1A. Previously, transcriptionally intact full-length HuCycT1 was found in nuclear dots/speckles (42). To check how Sp1A-competent forms of HuCycT1 might behave, we visualized using confocal microscopy to identify the subcellular location of seven HA-tagged mutants. Intriguingly, both highly active, (HuCycT1 (1–726),
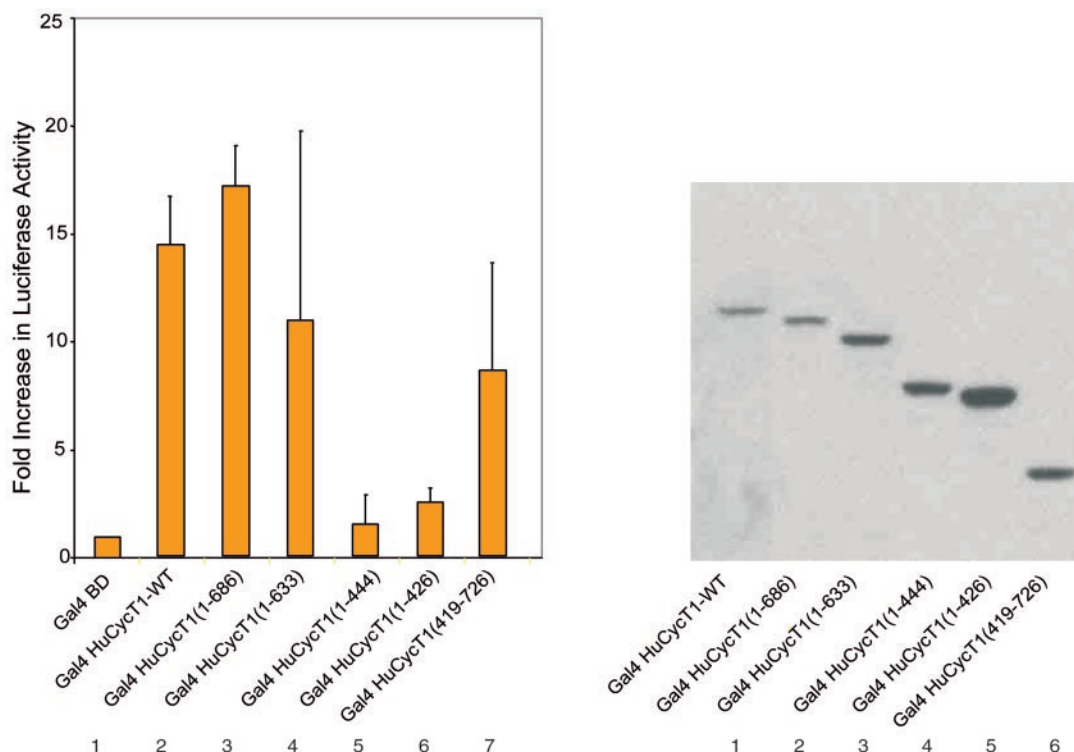
FIG. 5. **CycT1 C-terminal amino acids 419 to 726 are sufficient for promoter activation.** *Left*, HeLa cells were transfected with G5–83HIV-Luc and the indicated wild type and deletion mutants of HuCycT1 fused to Gal4BD. Luciferase was assayed 48 h later. *Right*, Western blot verification of comparable expression of transfected DNAs.

HuCycT1 (1–633), HuCycT1(240–726, not shown), and HuCycT1 (300–726)) and moderately active (HuCycT1 (419–726) and HuCycT1 (500–726)) cyclin T1s stained identically in nuclear speckles (Fig. 6). On the other hand, Sp1A-inactive HuCycT1 (1–444) and HuCycT1 (1–419), shown elsewhere to support P-TEFb/Tat/TAR function (24, 39), were not in speckles and stained predominantly cytoplasmic. To the extent that nuclear speckling might be a requisite for transcriptional activity, the divergent patterns suggest that Sp1A-active portion of cycT1 dictates such physical localization.

### DISCUSSION

HIV-1 expression occurs in two phases. After integration of the provirus into cellular chromosome, basal transcription from the LTR must initially drive the synthesis, however inefficiently, of a small amount of full-length 9.6-kb mRNA that provides for the synthesis of Tat. Tat then activates the LTR through TAR RNA (2) to further increase processive transcription. Over the past few years, P-TEFb (reviewed in Ref. 43) has emerged as an important cofactor for Tat/TAR-activated phase of LTR transcription. Thus, we understand the P-TEFb/Tat/TAR ternary complex modulates transcriptional elongation by hyperphosphorylation of RNAP II CTD (12, 14). Interestingly, although P-TEFb is present in elongation complexes, before the synthesis of TAR RNA, it is also found in HIV-1 LTR-engaged preinitiation complexes (20). Currently, despite much learned about Tat/TAR interactions with elongation-associated P-TEFb, little is known regarding how P-TEFb enters preinitiation complexes and the role that it might play in such moieties.

Here, we present evidence that P-TEFb may be recruited into preinitiation complexes through physical association of its cycT1 subunit with DNA-bound Sp1. Sp1 is a glutamine-rich promoter-proximal activator that interacts with TFIID to stabilize preinitiation complexes (44). We found that the 179-amino acid Sp1A domain that contains its glutamine-rich se-

quence is sufficient for physical (Fig. 3*C*) and functional (Figs. 3*B* and 4*A*) interaction with cycT1. Surprisingly, the portion of cycT1 delineated to cooperate with Sp1A mapped to a short, ~419–633-amino acid C-terminal stretch (Figs. 4*A* and 6). This Sp1A-active region resides outside of the cycT1 cyclin homology box (*i.e.* amino acids 1–290) and associates with neither cdk9 nor Tat. Thus, unlike TAR RNA-bound P-TEFb, DNA-bound cycT1-Sp1A activity apparently does not use cdk9. Although provocative, this unexpected conclusion is consistent, in part, with findings that Sp1-driven transcription can occur through a CTD-kinase independent mechanism (45, 46) and with evidence that productive and processive transcriptional elongation from the HIV-1 LTR can proceed without cdk9 (47). Currently, we do not rule out that in the absence of cdk9, other kinases such as CDK2 (48) may be involved.

It has been suggested elsewhere that cycT1 might be recruited to the HIV-1 preinitiation complexes through binding to NF-κB and contact with RNAP II CTD (49, 50). Although we have not directly examined interactions between NF-κB and cycT1, several observations seemingly disfavor this interpretation. First, the natural phylogeny of primate lentiviral LTRs is such that many simian LTRs have either single or no NF-κB binding sites (Fig. 1*A*). Thus, recruitment of cycT1 by NF-κB cannot be a generally conserved mechanism for all primate lentiviruses. Second, our linker-scanning analyses (Fig. 2*A*) showed that Sp1 is required, whereas NF-κB is not, for DNA-targeted cycT1 activity. Other studies (40, 51) have also independently suggested this Sp1-requirement. Hence, although we do not exclude that NF-κB might recruit cycT1 to HIV-1 DNA, our current results show that NF-κB is unnecessary, whereas Sp1 is necessary and sufficient. Intriguingly, in the two studies that proposed NF-κB recruitment of cycT1, presence of Sp1-binding sites in the reporter constructs were required for such activity (49, 50). Because NF-κB binds the N-terminal cyclin homology domain of cycT1 (49) and Sp1 binds
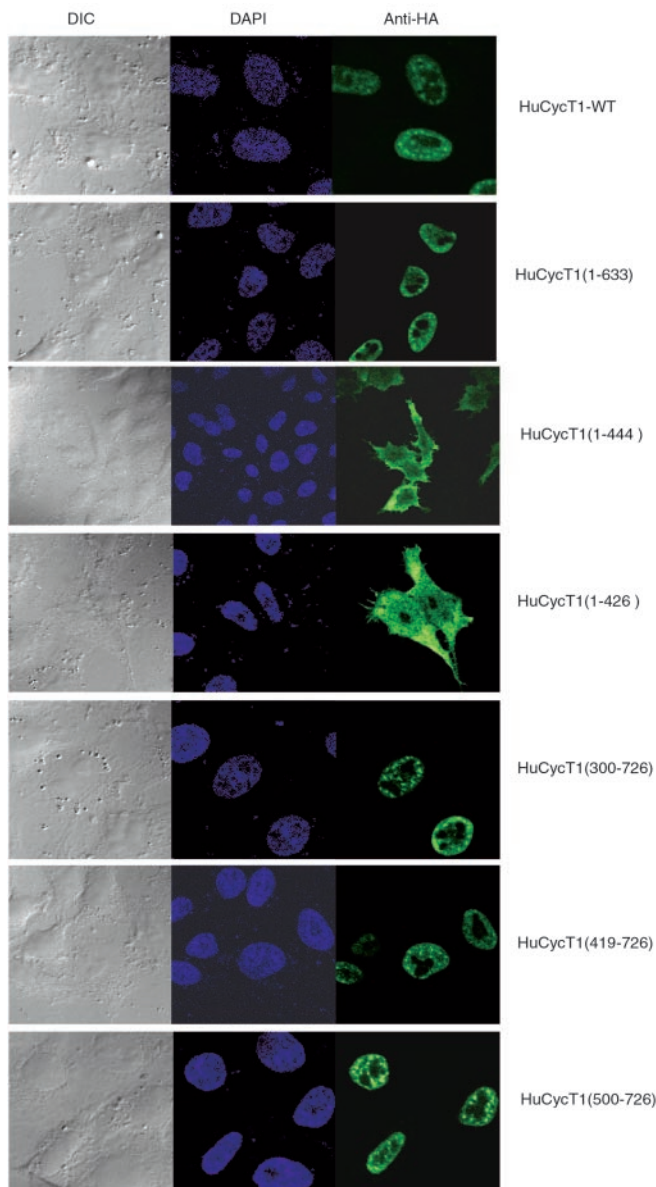
FIG. 6. **Localization of Sp1A-active cycT1 to nuclear speckles.** HeLa cells were transfected with the indicated HA-tagged HuCycT1. Cells were incubated with anti-HA and stained with anti-mouse conjugated to Alexa Fluor 488 (Molecular Probes). Nuclei were stained with 4,6-diamidino-2-phenylindole (Molecular Probes). Light field (*DIC* (differential interference contrast)) is left; 4,6-diamidino-2-phenylindole (*DAPI*) is middle; anti-HA is right.

the C terminus of cycT1, it is possible, however, that in the natural context of the HIV-1 LTR, both factors cooperate in high affinity capture of cycT1. DNA-tethered cycT1 may then subsequently contact RNAP II CTD directly (50).

In addition to binding Sp1, the C terminus of cycT1 has another important activity. It is well known that transcription factors and actively transcribing RNAP II physically localize into nuclear foci with speckled patterns (52, 53). Accordingly, a prerequisite for P-TEFb-activity is its entry into nuclear speckles (42, 54). *A priori*, one might suppose that P-TEFb is directed to such a locale through the N-terminal Tat/TAR associative domain of its cycT1 component. Surprisingly, our data show that the Sp1A-interactive C terminus (500–726 amino acids) of cycT1 dictates its localization into nuclear dots (Fig. 6). The N terminus (*e.g.* 1–444 amino acids) of cycT1, which binds cdk9, has no such function (Fig. 6). Hence, the first step of P-TEFb function, its constitutive (or Sp1-associated) migration to nu-

clear speckles, seems to be determined through its C terminus.

DNA recruitment of cycT1 by Sp1 serves also to address the long-standing observation that Tat function and HIV-1 transcription are apparently defective in rodent cells (29, 55, 56). Two recent studies have unexpectedly shown that HIV-1 is both transcriptionally intact and replication competent in rat cells (21, 22), despite the fact that rat cycT1 lacks the human specific cysteine 261 residue (29, 55–57) necessary for forming a P-TEFb/Tat/TAR complex. Accordingly, in rat cells, the prototypic elongation-associated P-TEFb/Tat/TAR complex cannot form. How then can HIV-1 transcription occur successfully in rat cells? Although we have not examined rat cycT1 directly, we find in human cells that MuCycT1 and HuCycT1 are equally active in their Sp1-dependent activity. Thus, DNA-targeted cycT1 does not need cysteine 261 for LTR expression. Intriguingly in rat cells, we have observed that Tat-independent activity of MuCycT1 is actually considerably higher than that of HuCycT1.[2] Conceivably, a rat-specific cell factor may enhance DNA-based Sp1-cycT1 interaction to sufficiently bypass the need for RNA-tethered P-TEFb/Tat/TAR. Indeed active P-TEFb at cellular promoters functions effectively without Tat or TAR (43). We have recently observed that, consistent with the idea that Tat/TAR may not be absolutely necessary for lentiviral LTR and consistent with the proposal of Bieniasz and Cullen (58), overexpression of either HuCycT1 or MuCycT1 fully activates the transcription and synthesis of viral proteins from a Tat(−) HIV-1 provirus (data not shown).

REFERENCES

1. Taube, R., Fujinaga, K., Wimmer, J., Barboric, M., and Peterlin, B. M. (1999) *Virology* **264,** 245–253
2. Berkhout, B., Silverman, R. H., and Jeang, K. T. (1989) *Cell* **59,** 273–282
3. Cujec, T. P., Okamoto, H., Fujinaga, K., Meyer, J., Chamberlin, H., Morgan, D. O., and Peterlin, B. M. (1997) *Genes Dev.* **11,** 2645–2657
4. Garcia-Martinez, L. F., Mavankal, G., Neveu, J. M., Lane, W. S., Ivanov, D., and Gaynor, R. B. (1997) *EMBO J.* **16,** 2836–2850
5. Parada, C. A., and Roeder, R. G. (1996) *Nature* **384,** 375–378
6. Herrmann, C. H., and Rice, A. P. (1995) *J. Virol.* **69,** 1612–1620
7. Laybourn, P. J., and Dahmus, M. E. (1990) *J. Biol. Chem.* **265,** 13165–13173
8. Marciniak, R. A., and Sharp, P. A. (1991) *EMBO J.* **10,** 4189–4196
9. Yang, X., Gold, M. O., Tang, D. N., Lewis, D. E., Aguilar-Cordova, E., Rice, A. P., and Herrmann, C. H. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 12331–12336
10. Zhu, Y., Pe'ery, T., Peng, J., Ramanathan, Y., Marshall, N., Marshall, T., Amendt, B., Mathews, M. B., and Price, D. H. (1997) *Genes Dev.* **11,** 2622–2632
11. Peng, J., Zhu, Y., Milton, J. T., and Price, D. H. (1998) *Genes Dev.* **12,** 755–762
12. Wei, P., Garber, M. E., Fang, S. M., Fischer, W. H., and Jones, K. A. (1998) *Cell* **92,** 451–462
13. Marshall, N. F., and Price, D. H. (1995) *J. Biol. Chem.* **270,** 12335–12338
14. Mancebo, H. S., Lee, G., Flygare, J., Tomassini, J., Luu, P., Zhu, Y., Peng, J., Blau, C., Hazuda, D., Price, D., and Flores, O. (1997) *Genes Dev.* **11,** 2633–2644
15. Fujinaga, K., Cujec, T. P., Peng, J., Garriga, J., Price, D. H., Grana, X., and Peterlin, B. M. (1998) *J. Virol.* **72,** 7154–7159
16. Zhou, Q., Chen, D., Pierstorff, E., and Luo, K. (1998) *EMBO J.* **17,** 3681–3691
17. Bieniasz, P. D., Grdina, T. A., Bogerd, H. P., and Cullen, B. R. (1998) *EMBO J.* **17,** 7056–7065
18. Lis, J. T., Mason, P., Peng, J., Price, D. H., and Werner, J. (2000) *Genes Dev.* **14,** 792–803
19. Kanazawa, S., Okamoto, T., and Peterlin, B. M. (2000) *Immunity* **12,** 61–70
20. Ping, Y. H., and Rana, T. M. (1999) *J. Biol. Chem.* **274,** 7399–7404
21. Reid, W., Sadowska, M., Denaro, F., Rao, S., Foulke, J., Jr., Hayes, N., Jones, O., Doodnauth, D., Davis, H., Sill, A., O'Driscoll, P., Huso, D., Fouts, T., Lewis, G., Hill, M., Kamin-Lewis, R., Wei, C., Ray, P., Gallo, R. C., Reitz, M., and Bryant, J. (2001) *Proc. Natl. Acad. Sci. U. S. A.* **98,** 9271–9276
22. Keppler, O. T., Yonemoto, W., Welte, F. J., Patton, K. S., Iacovides, D., Atchison, R. E., Ngo, T., Hirschberg, D. L., Speck, R. F., and Goldsmith, M. A. (2001) *J. Virol.* **75,** 8063–8073
23. Bieniasz, P. D., Grdina, T. A., Bogerd, H. P., and Cullen, B. R. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 7791–7796
24. Garber, M. E., Wei, P., KewalRamani, V. N., Mayall, T. P., Herrmann, C. H., Rice, A. P., Littman, D. R., and Jones, K. A. (1998) *Genes Dev.* **12,** 3512–3527
25. Berkhout, B., and Jeang, K. T. (1992) *J. Virol.* **66,** 139–149
26. Zeichner, S. L., Kim, J. Y., and Alwine, J. C. (1991) *J. Virol.* **65,** 2436–2444
27. Southgate, C. D., and Green, M. R. (1991) *Genes Dev.* **5,** 2496–2507
28. Kibler, K. V., and Jeang, K. T. (2001) *J. Virol.* **75,** 2161–2173
29. Chen, D., Fong, Y., and Zhou, Q. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,**

[2] V. S. R. K. Yedavalli, unpublished observations.

2728–2733

30. Fu, T. J., Peng, J., Lee, G., Price, D. H., and Flores, O. (1999) *J. Biol. Chem.* **274,** 34527–34530

31. Zhou, M., Halanski, M. A., Radonovich, M. F., Kashanchi, F., Peng, J., Price, D. H., and Brady, J. N. (2000) *Mol. Cell. Biol.* **20,** 5077–5086

32. Jeang, K. T., and Gatignol, A. (1994) *Curr. Topics Microbiol. Immunol.* **188,** 123–144

33. Kadonaga, J. T., Carner, K. R., Masiarz, F. R., and Tjian, R. (1987) *Cell* **51,** 1079–1090

34. Kadonaga, J. T., Courey, A. J., Ladika, J., and Tjian, R. (1988) *Science* **242,** 1566–1570

35. Jones, K. A., Kadonaga, J. T., Luciw, P. A., and Tjian, R. (1986) *Science* **232,** 755–759

36. Chun, R. F., Semmes, O. J., Neuveut, C., and Jeang, K. T. (1998) *J. Virol.* **72,** 2615–2629

37. Lin, X., Taube, R., Fujinaga, K., and Peterlin, B. M. (2002) *J. Biol. Chem.* **277,** 16873–16878

38. Chen, D., and Zhou, Q. (1999) *Mol. Cell. Biol.* **19,** 2863–2871

39. Ivanov, D., Kwak, Y. T., Nee, E., Guo, J., Garcia-Martinez, L. F., and Gaynor, R. B. (1999) *J. Mol. Biol.* **288,** 41–56

40. Fraldi, A., Licciardo, P., Majello, B., Giordano, A., and Lania, L. (2001) *J. Cell. Biochem.* **81,** 247–253

41. Majello, B., Napolitano, G., Giordano, A., and Lania, L. (1999) *Oncogene* **18,** 4598–4605

42. Marcello, A., Cinelli, R. A., Ferrari, A., Signorelli, A., Tyagi, M., Pellegrini, V., Beltram, F., and Giacca, M. (2001) *J. Biol. Chem.* **276,** 39220–39225

43. Price, D. H. (2000) *Mol. Cell. Biol.* **20,** 2629–2634

44. Gill, G., Pascal, E., Tseng, Z. H., and Tjian, R. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 192–196

45. Gerber, H. P., Hagmann, M., Seipel, K., Georgiev, O., West, M. A., Litingtung, Y., Schaffner, W., and Corden, J. L. (1995) *Nature* **374,** 660–662

46. Chun, R. F., and Jeang, K. T. (1996) *J. Biol. Chem.* **271,** 27888–27894

47. West, M. J., and Karn, J. (1999) *EMBO J.* **18,** 1378–1386

48. Nekhai, S., Zhou, M., Fernandez, A., Lane, W. S., Lamb, N. J., Brady, J., and Kumar, A. (2002) *Biochem. J.* **364,** 649–657

49. Barboric, M., Nissen, R. M., Kanazawa, S., Jabrane-Ferrat, N., and Peterlin, B. M. (2001) *Mol. Cell* **8,** 327–337

50. Taube, R., Lin, X., Irwin, D., Fujinaga, K., and Peterlin, B. M. (2002) *Mol. Cell. Biol.* **22,** 321–331

51. Napolitano, G., Majello, B., Licciardo, P., Giordano, A., and Lania, L. (2000) *Gene* **254,** 139–145

52. Wansink, D. G., Schul, W., van, d. K., I, van Steensel, B., van Driel, R., and de Jong, L. (1993) *J. Cell Biol.* **122,** 283–293

53. Jackson, D. A., and Cook, P. R. (1993) *J. Cell Sci.* **105,** 1143–1150

54. Herrmann, C. H., and Mancini, M. A. (2001) *J. Cell Sci.* **114,** 1491–1503

55. Fujinaga, K., Taube, R., Wimmer, J., Cujec, T. P., and Peterlin, B. M. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 1285–1290

56. Garber, M. E., and Jones, K. A. (1998) *Cold Spring Harbor Symp. Quant. Biol.* **63,** 371–380

57. Zhang, J., Tamilarasu, N., Hwang, S., Garber, M. E., Huq, I., Jones, K. A., and Rana, T. M. (2000) *J. Biol. Chem.* **275,** 34314–34319

58. Bieniasz, P. D., and Cullen, B. R. (2000) *J. Virol.* **74,** 9868–9877